Title: Lighting JeWells: HCS-SPIM platform for organoids allowing morphogenesis analysis based on machine learning.

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Turning organoids into an impactful translational technique includes be able to generate and to assess organoids that develop robustly and to physiologically relevant architecture. Nevertheless, the lack of high-throughput 3D imaging methods precludes any quantitative comparisons and statistical analysis at high content, mandatory to describe the complexity of such multicellular 3D objects. We engineered a versatile High Content Screening (HCS) platform allowing to streamline all the steps of organoids culture with high resolution 3D imaging to exploit their whole potential in morphogenesis understanding. Our approach comprises a new generation of versatile scaffolding cell culture multiwell chips with embedded optical components (= lighting JeWells) that enables fast 3D imaging using the soSPIM technology [1]. The high surface density of JeWells meets HCS standards: we can generate more than hundred organoids in a surface equivalent to a single well of a 384 wellplate. This platform allows us to follow, during weeks, the morphogenesis of living organoids thanks to non-toxic light sheet microscopy based fluorescent imaging (validated on hESC, hIPSC and primary cells). The large number of 3D images generated can then be used to train convolutional neural networks to precisely detect and quantify subcellular and multicellular features, such as mitotic and apoptotic events, or segment multicellular structures (rosettes), and classify whole organoid morphologies. Such a combination of high resolution 3D microscopy techniques with HCS and machine learning approaches allow us to quantitatively describe the morphogenesis of hundreds of living organoids correlated with phenotypic characterization to decipher mechanisms involved in human developmental biology, tissue pathology, and to enrich drug discovery pipeline.

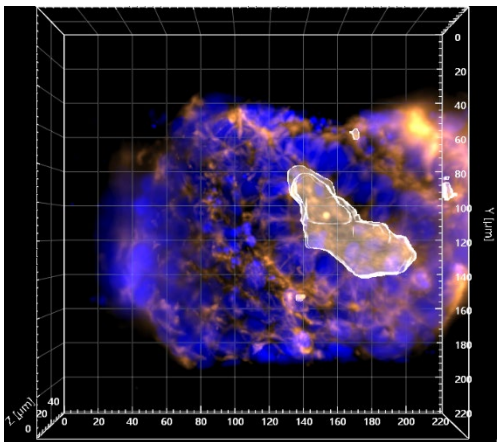

Figure 1: AI based 3D segmentation of a rosette in a neuroectoderm organoid.

[1] R. Galland, G. Grenci, A. Aravind, V. Viasnoff, V. Studer, and J.-B. Sibarita, "3D high- and super-resolution imaging using single-objective SPIM," *Nat. Methods*, vol. 12, no. 7, pp. 641–644, 2015, doi: 10.1038/nmeth.3402.